

For J.F. Crow

THE UNORTHODOX MATING BEHAVIOR OF BACTERIA.

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Looking at recent papers of bacterial genetics I am surprised at being still able to recognize the names of some of the markers and of the strains of Escherichia coli K-12. So much has happened since that exciting time. E.coli is well on its way to become one of those few organisms of which we know the whole genome, an incredible progress from what was known around 1950 when we knew a map of less than a dozen markers. Starting in 1941 bacteria had become my major interest, and in 1948 I gave a paper at the international congress of Genetics in Stockholm on cross resistance to radiation and to nitrogen mustard in E.coli, based on work done earlier in Milan with Niccolo' Visconti. In 1948 I had a scholarship with Kenneth Mather at the John Innes Horticultural Institution, then at Merton and directed by C.D. Darlington. Italy was then, and remained for decades, a scientific desert with a few oases. I was lucky to have found after much search one of these oases, with Adriano Buzzati-Traverso as my professor. 1948 was the first time I was able to go abroad, a major success in post-war Italy, and I enjoyed enormously drinking directly at one of the original fountains of genetic and statistical knowledge.

It was in that eventful summer 1948 that I had the surprise, just after introducing myself to R.A. Fisher at the Stockholm international Congress, of being offered a job in his laboratory. Fisher was probably one of the very few readers of the paper by Joshua Lederberg, appeared in 1947 on Genetics, who understood it in its entirety and believed it. The popes and cardinals of bacteria and phage who listened to the first communication by Lederberg at the famous 1946 Cold Spring Harbor symposium were at the beginning sceptical of the E.coli K-12 crosses. Unlike them, Fisher immediately developed enthusiasm for K-12 genetics. He obviously was not scared by what Jim Watson in the Double Helix called the "rabbinical complexity" of Joshua's papers. His main experimental interest was crossing-over, which he studied mostly on mice. His lab was full of these smelly animals, his garden of various experimental plants, including Mendel's peas. His hope was that E.coli might become an excellent organism for the study of crossing-over. Fisher also tried, unsuccessfully, to initiate me to mouse genetics.

I immediately accepted Fisher's offer and started working in Cambridge, 44 Storey's Way - the address of the old Genetics Dept there - on Oct. 1st 1948. My lab was carved out of the tea room and I must confess I chose the equipment not so much on the basis of price or reliability, but simply from firms which offered to make it available sooner. In any case, there was very little

difference in prices, and reliability was hard to guess; moreover, a bacteriological laboratory had really simple equipment at the time. K-12 strains were sent by the Lederbergs and I was beginning to cross them in February, 1949. It was perfectly easy to repeat the original experiments; people did not believe them because they did not try them. The scepticism around me was incredible. To classical bacteriologists we - the very few bacterial geneticists, to be counted on one hand - were lunatics. Bacteriologists had been taught that bacteria have no nucleus or chromosomes and besides, very few of them had clear ideas of mendelism and, in particular, of recombination. Geneticists of fungi, like D.G. Catcheside and Guido Pontecorvo were not so sceptical. Guido had developed a procedure for selecting recombinants in Ascomycetes very similar to the prototroph technique which permitted to Lederberg to show bacterial recombination: mixing different mutants on a medium in which neither parent could grow, but a recombinant would. On Guido's invitation, I gave in 1950 in Glasgow a demonstration of bacterial recombination to bacteriologists. At the time, it was customary to wash three times suspensions of the parent bacteria before plating them on minimal medium (without the nutritional supplements necessary for growth of the parental mutant strains), and the many bacteriologists who came to see had to stand for a long time during these very simple but lengthy operations. I also kept them at some distance for fear of contaminations.

Recombination was, at the beginning a very rare event: it stopped being rare when I found a mutant strain which I called Hfr for high frequency of recombination. It was an accident that happened in 1949 having selected mutants resistant to nitrogen mustard and to radiation. The first two resistant mutants, which had undergone a rather heavy treatment in the process of selection for nitrogen mustard resistance proved to be exceptional in their mating behavior: one was Hfr, and it showed immediately its remarkable mating ability, which was higher than that of normal crosses by a factor of 1000 or more. I repeated the experiment two more times before believing it. Another was, as I later proved, an F- mutant of an F+ strain.

Hfr was exceptionally interesting but the biology of mating was difficult to understand. There was nothing to be clearly seen microscopically on a plate or in mixed cultures; no distinguishable zygotes were formed. It was only in 1954 that Lederberg first proved by micromanipulation experiments that when mating took place there was something - an invisible "rope" - holding a male and a female together in a drop of saline, though at some distance one from the other. Electron microscopy was for a long time negative or unclear. The genetics of the Hfr crosses was difficult to understand, not surprisingly (in retrospect). I wanted to publish something only when I felt I did understand the phenomenon, and thus I published almost nothing about the finding of Hfr, except a short mention in an Italian journal in 1950. A year or so later, another independent Hfr turned out spontaneously in an old culture in Great Britain and was studied by W.

Hayes. The two Hfrs are still around, and are called with our two names or simply their initials, HfrC, HfrH. Much later, many more independent Hfrs were obtained. Each Hfr seems to have unique properties.

Since October 1950 I had returned to Milan, back to the laboratory of the Istituto Sieroterapico Milanese where I had started working in 1945 after the end of the war. Although this was a pharmaceutical firm, I was able to continue my genetic research on a part time basis. I undertook the examination of other fertility mutants which proved easier. The original K-12 strain is capable of mating with itself but at a low frequency. I found several independent mutants had lost the capacity to mate with themselves. To show it, I had to develop new biochemical mutants that would make it possible to test if a strain could or not mate with itself. Self-sterile strains are called F-. F+ is the original K-12 strain, fertile at a low rate with all F- strains, and at an even lower rate with itself. Hfr is a mutant of F+ which has a high frequency of recombination. While the progeny of F+ x F- crosses was consistently F+ (except with some special F- strains, a phenomenon I never came around to publish), that of Hfr x F- was consistently F-. But short term mixture of F+ and F- cells, allowing contact of them, could pass the F+ property to F- cells with high probability.

While I was doing these experiments I was in correspondence with Joshua Lederberg, and wrote to him about these findings. He and Esther had had very similar results, and we decided to publish them together: one joint paper was sent to Genetics, another to Journal of General Microbiology. The Lederbergs and I had never met, and did so only when I could go to Madison, Wis. in 1954, thanks to a Rockefeller Fellowship that allowed me to work with them for three months. It was a strange but pleasant experience to write papers with people known only through air mail.

In England I had met Bill Hayes. I happened to give him the first E.coli K-12 strains, and show him the crossing and scoring techniques in the practicals of a course which was held at Cambridge. Bill and I also corresponded, though more rarely. He once wrote me on F+ and F- of which I had written to him: "I guess one can pass the F+ property by infection to an F-". Both the Lederbergs and I had independently found this, and I hastened to write to him that he would find the experiment works quite well. He later told me that he was quite shocked when he received my answer because he also had meanwhile done the experiment. It was planned the Hayes paper would appear in the same issue of Journal of General Microbiology as ours, as it did.

In 1952 I had a student of Kenneth Mather, John L. Jinks as guest in my Milan laboratory. It was at the time clear to us that the Hfr x F- cross yielded F- progeny, as I said above. But we now found that the cross of HfrC with an F- did generate some Hfr progeny, clearly in linkage with a Galactose marker which was rarely segregating. Results thus could be summarised by saying that F was an infectious particle which could be easily transmitted by cell-to-cell contact, and showed no indication of linkage to other markers, but in some conditions it would become

irreversibly part of the bacterial chromosome at a specific site, losing the capacity to infect by cell-to-cell contact but acquiring that of high frequency of recombination. We communicated this finding to the Bellagio 1953 International Congress of Genetics together with the first information on recombination and fitness. This was one of the original purposes of my work in Fisher's department; for instance, all possible parental combination of three markers were tried to test for effects of markers viability. Until the mechanism of fertility became clear, research on E.coli recombination had proved entirely frustrating. Jim Watson, who was then at Cambridge spent a few days in Milan in 1952 to see my recombination data. He was convinced that a three chromosome theory could explain the observations. He offered to write a paper together (which he later published with Hayes) but I was not persuaded by the theory and declined.

It became progressively clear that there were some phenomena which could be interpreted on the basis of breaks and that a specific chromosome region was contributed only by females (F-) parents. This made the results of recombination difficult to understand. It took a long time for Jinks and myself to agree on the formal interpretation of recombination data, and it was only in 1956 that we were able to publish a joint manuscript. The formal interpretation of detailed recombination results published by us and by others was, I think, correct to this day, and a tour-de-force of recombination analysis. It showed how difficult it would have been to use the system of E.coli recombination for the quantitative study of crossing over, as Fisher was hoping to do. Nevertheless, Fisher followed with great interest and full open-mindedness the unexpected results that were coming out of bacterial crosses, and was more flexible than I in accepting the unorthodox behaviors of bacteria.

My position in the Istituto Sieroterapico Milanese was not ideal for keeping up with the explosion of research on E.coli genetics. Beginning in 1952 I started flirting with human genetics while lecturing part-time at the University of Parma and slowly left bacteria. The last Petri dish I touched must have been in 1960, working with Joshua and Esther Lederberg in Stanford on the effects of streptomycin on the phenotype of bacterial mutants, a very interesting phenomenon we, as well as Luigi Gorini independently observed. Conversion to human genetics provided a completely different outlet for my scientific interests, replacing work on the laboratory bench with statistical and theoretical analysis.

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